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HPLC analysis of camptothecin content in various parts of *Nothapodytes foetida* collected on different periods

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ABSTRACT

Objective: To investigate the content of topoisomerase I-DNA inhibitor alkaloid camptothecin (CPT) from various parts of *Nothapodytes foetida* (*N. foetida*) collected from the month of October to February. **Methods:** The content of CPT was quantified in the methanolic extract of various parts of *N. foetida* using high performance liquid chromatography (HPLC). Quantification was performed with the regression analysis and the method was validated as per ICH guidelines. **Results:** The results revealed that maximum concentrations of camptothecin were found in root (2.62%) collected in the month of February followed by fruits (January, 1.22%), stem (January, 0.81%) and leaves (February, 0.70%). Roots were found to have 3-fold higher concentration of CPT than the leaves and stem, while the fruits showed 2-fold higher concentration. Maximum concentration of camptothecin in fruits was observed in month of January, when they were not fully mature, which was 2-fold higher than that of young and fully mature fruits. **Conclusions:** These findings indicate that the synthesis of CPT differs in different parts of *N. foetida* and the content varies periodically.

1. Introduction

Nothapodytes foetida (*N. foetida*) (Icacinaceae) is a rich source of the potent alkaloid camptothecin (CPT) (Figure 1) The cellular target of camptothecin is DNA topoisomerase I, and numerous analogs have been synthesized as potential therapeutic agents. CPT inhibits the replication of human immuno deficiency virus (HIV) *in vitro* and is also shown to be effective in the complete remission of lung, breast, uterine and cervical cancer. CPT was first isolated from a Chinese deciduous tree *Camptotheca acuminata*, later it was isolated from a variety of plant species including *Merriliodendron megacarpum* and *N. foetida*, *Ophiorrhiza mungos* and *Ervatamia heyneana* and *Mostuea brunonis*. Among these, the maximum concentration of CPT has been reported from *N. foetida*[1-5]. Quantification of CPT content from *N. foetida* by different analytical methods like high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), desorption electrospray ionization mass spectrometry (DESI-MS) and proton nuclear magnetic resonance spectroscopy (1H-NMR) methods has been reported[1,5-10]. The aim of the present study was to quantify CPT concentrations in various parts of *N. foetida* collected in different months by HPLC method.

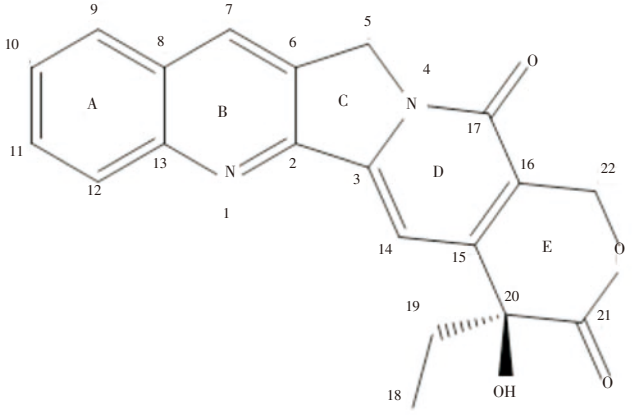

Figure 1. Chemical structure of CPT.

2. Materials and methods

2.1. Plant materials

Plant materials of *N. foetida* were obtained from the Mahabaleshwar region of Maharashtra, India, from the month of October to February. The samples were

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authenticated by Botanical Survey of India (BSI) and voucher specimen (NNASP1) was kept at departmental herbarium of BSI. Drug materials were powdered and stored at 25 ℃ in an airtight container. Reference standard of CPT (purity 98% w/w) was purchased from Hi media (Mumbai, India). Solvents were purchased from Merck, Mumbai, India. All chemicals used were of analytical grade.

2.2. Sample preparation and extraction

Plant materials (roots, stems, leaves and fruits) of *N. foetida* were collected and dried at 55 ℃ in an air dryer for 48 h. Dried materials were powdered with a Wiley mill (Model 4276-M, Thomas Scientific, USA) to pass a 20 mesh sieve and stored in sealed plastic bags. Dried materials (500 mg) were weighed in 5 mL volumetric flask, percolated with 5 mL of MeOH and vortexed for 2 min followed by sonication (33 MHz, Roop Telesonic, India) at room temperature for 15 min. The process was repeated twice for complete extraction. After sonication, methanolic extracts were combined and evaporated to dryness in vacuo. For determination of CPT content, the concentrate was transferred into polypropylene micro-centrifuge tubes, mixed with HPLC grade MeOH (1 mL), vortexed for 20 s followed by centrifugation at 5 000 rpm for 10 min. The clean supernatants were applied directly onto HPLC.

2.3. High performance liquid chromatography

Quantification of CPT was performed by following the method of Fulzele and Satdive[5]. Isocratic analytical HPLC assay was performed on a Jasco 900 instrument and 5 μL of supernatant extracts was loaded onto octadecyl silane (ODS) (5 μm; Inertsil) column (150 × 4.6 mm). Acetonitrile: water (45:55) was eluted at a flow rate of 1 mL/min and the alkaloids were detected at 366 nm by UV detector (UV-975, Jasco). The peak areas corresponding to CPT were integrated by comparison with external standard calibration curves. HPLC assay of different extracts yielded chromatograms with a retention time 4.1 min for CPT. Validation of quantitative method was performed with samples for three times. The results of the three injections from the same samples at the five concentrations (10-50 μg/mL) showed similar retention time. The standard deviation proved that the accuracy and reproducibility was excellent.

2.4. Preparation of standard solution of CPT

A stock solution of CPT was prepared by dissolving 5 mg of accurately weighed CPT in chloroform-methanol mixture (3:1) and making up the volume to 5 mL with methanol. From this stock solution, standard solutions of 10 μg/mL to 50 μg/mL were prepared by transferring aliquots (0.1 to 0.5 mL) of stock solution to 10 mL volumetric flasks and adjusting the volume with methanol.

2.5. Calibration curve for CPT

20 μL of standard solutions of CPT was injected in triplicate in column. The peaks were detected at 360 nm. The peak areas were recorded. Calibration curves of CPT were prepared by plotting peak area vs. concentration with correlation coefficient R^2=0.998 (Y=113140X+453.5).

3. Results

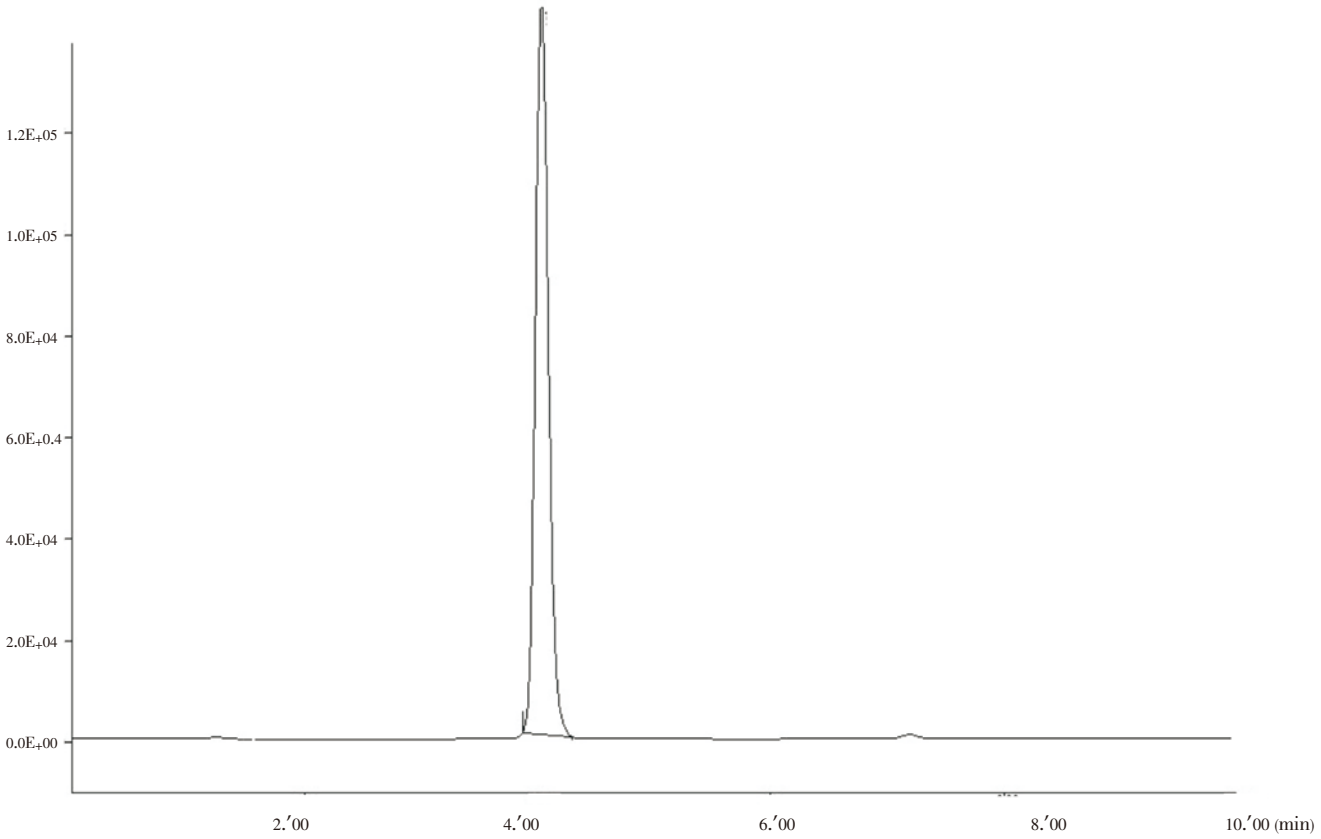

Figure 2. HPLC chromatogram of authentic CPT.

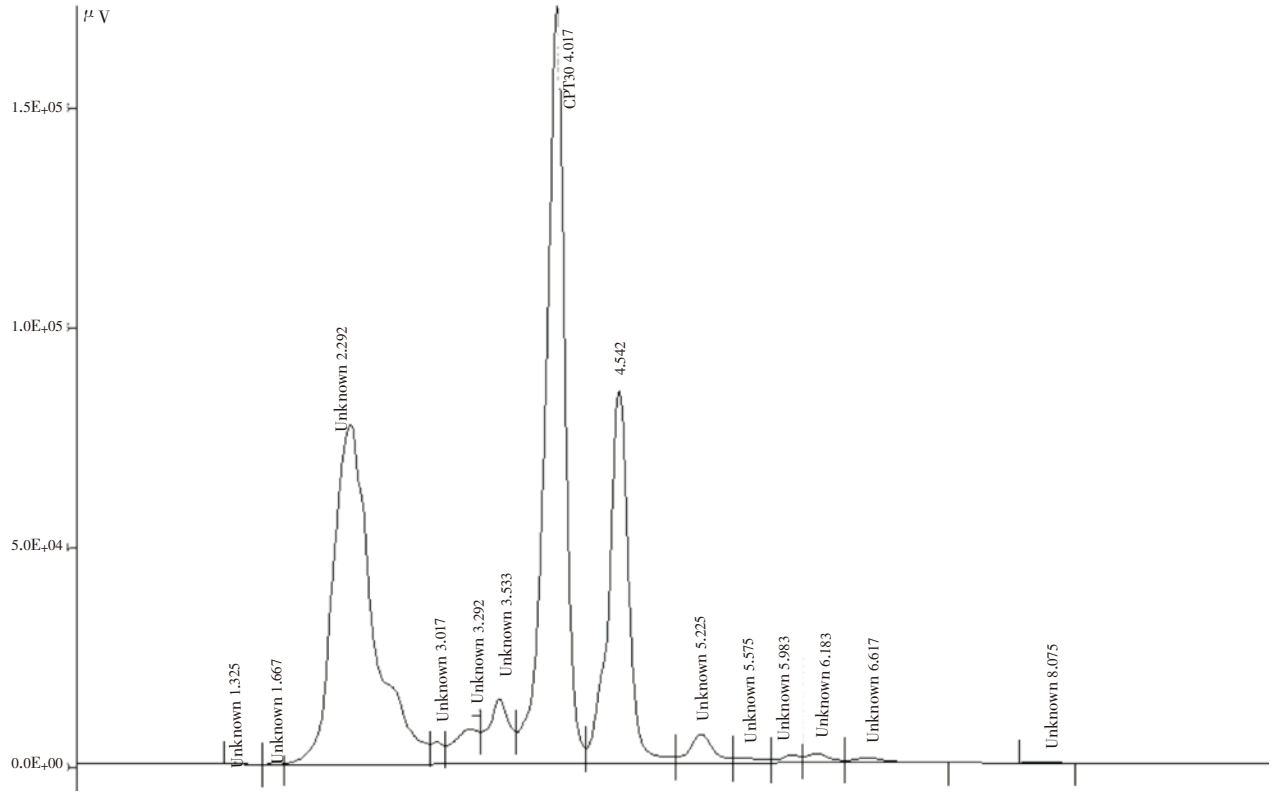

Figure 3. HPLC chromatogram of root sample of *N. foetida* spiked with CPT.

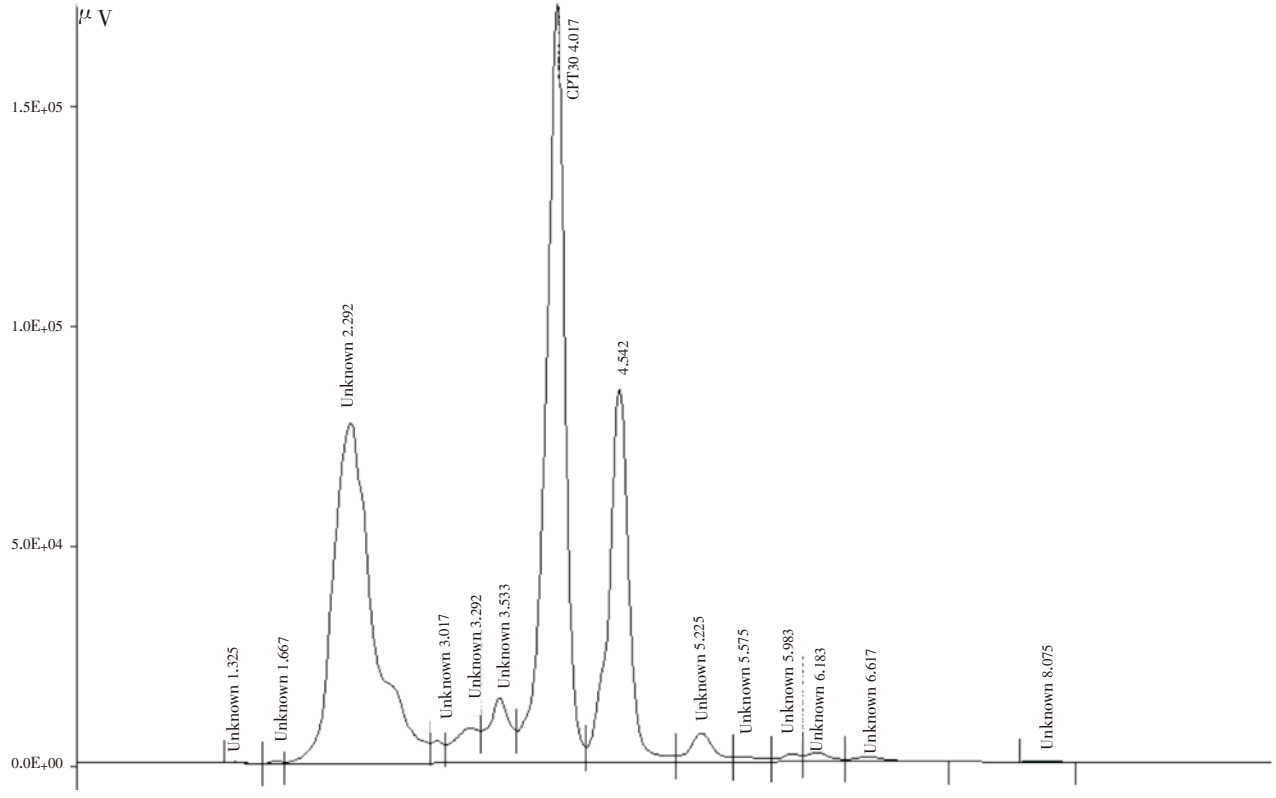

Figure 4. HPLC chromatogram of stem sample of *N. foetida* spiked with CPT.

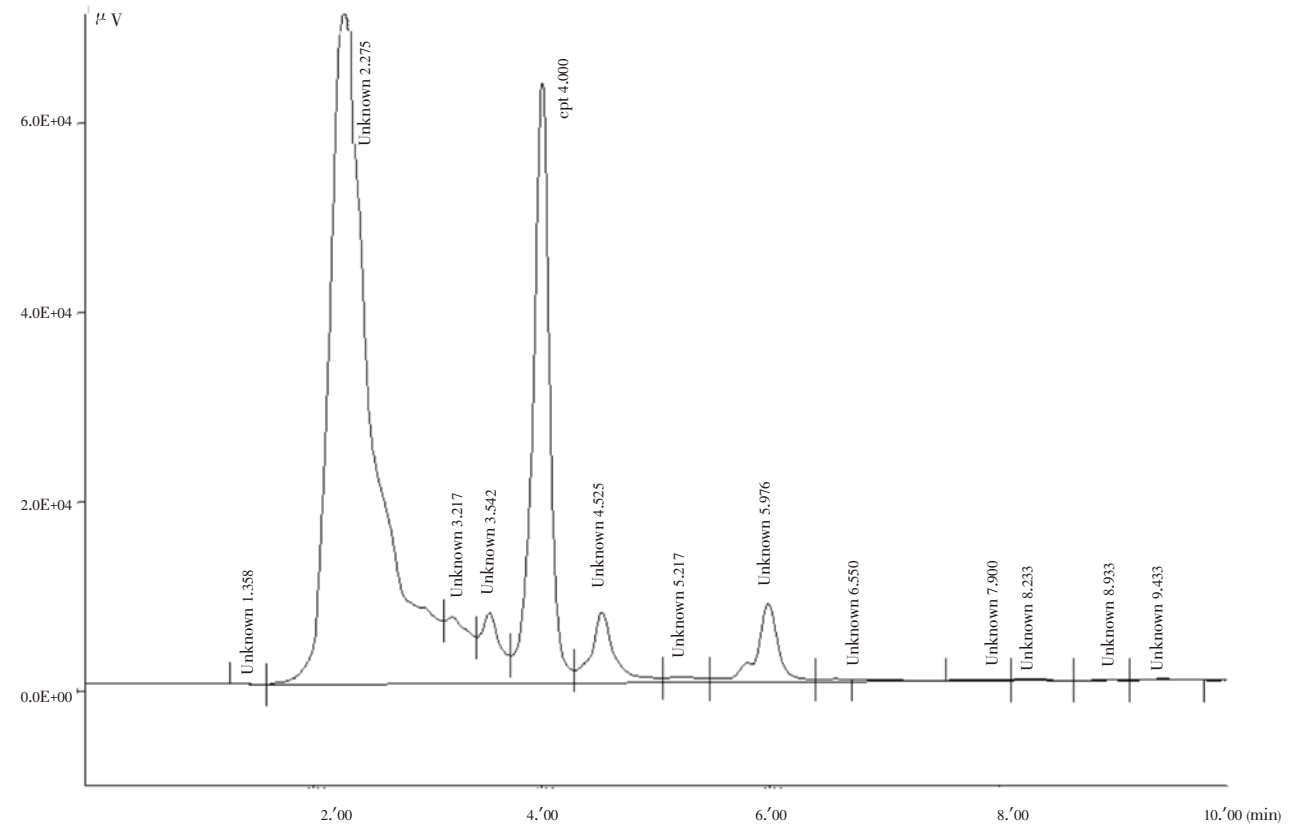

Figure 5. HPLC chromatogram of leaves sample of *N. foetida* spiked with CPT.

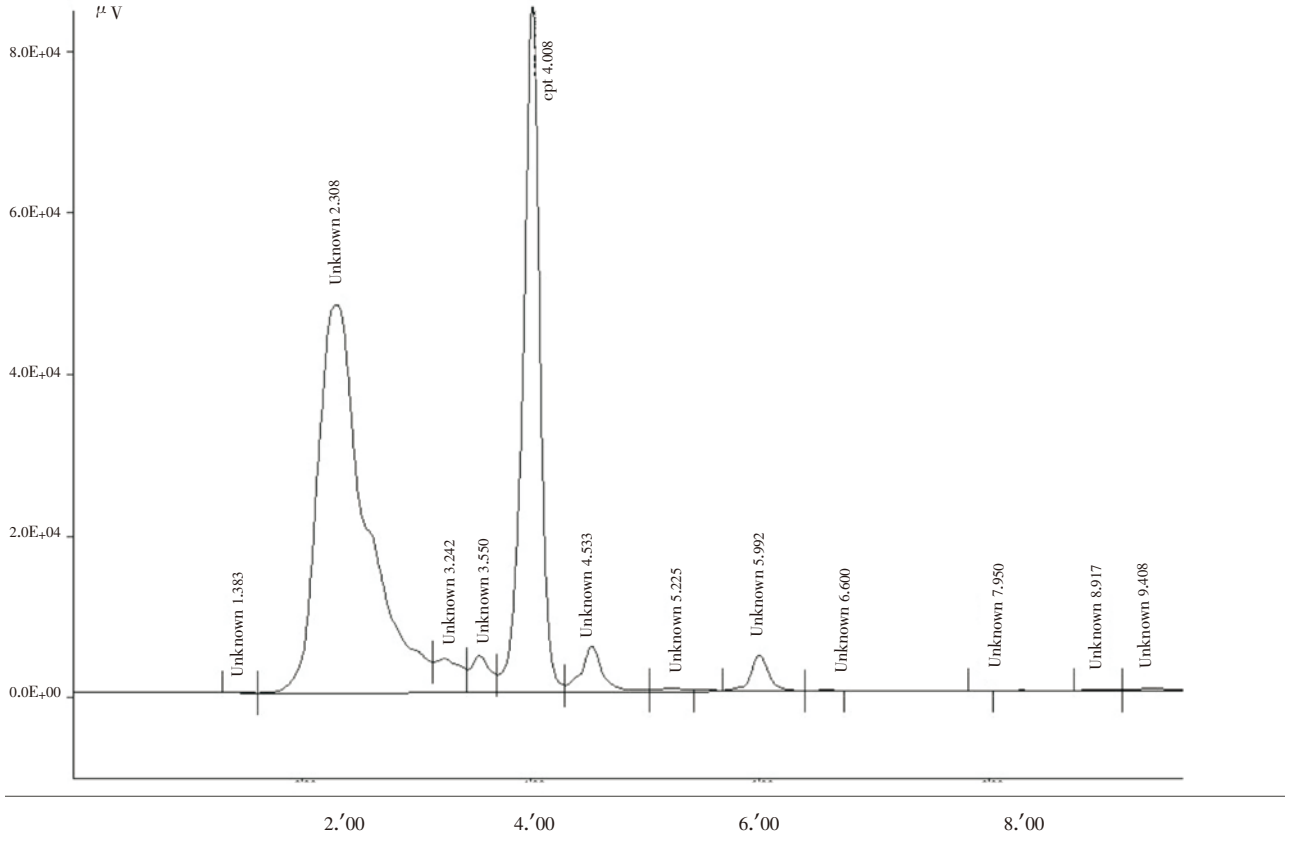

Figure 6. HPLC chromatogram of fruits sample of *N. foetida* spiked with CPT.

The HPLC analysis resulted in a sharp peak of authentic CPT at the retention time of 4.1 min (Figure 2). The contents of CPT in various parts of *N. foetida* collected from the month of October to February are shown in Table 1.

The order of CPT content was observed as maximum in root followed by fruits, then in stems and minimum in leaves. The maximum concentration of CPT was obtained in root (2.62%) collected in the month of February followed by fruits collected in the month of January (1.22%). Stem showed 0.81% of CPT in the month of January while the leaves contain 0.70% CPT in February. There was a 3-fold higher concentration of CPT observed in roots than the leaves and stem, while 2-fold higher concentration was observed in the fruits. The chromatogram for the root, stem, leaves, and fruits are shown in Figure 3-6.

Table 1

CPT contents in various parts of *N. foetida* collected from the month of October to February.

Period	CPT (% w/w)			
	Root	Stem	Leaf	Fruit
October	0.200 ± 0.020	0.340 ± 0.010	0.410 ± 0.100	0.510 ± 0.050
November	0.210 ± 0.030	0.360 ± 0.030	0.520 ± 0.300	0.620 ± 0.001
December	0.930 ± 0.030	0.230 ± 0.010	0.200 ± 0.040	0.150 ± 0.040
January	0.680 ± 0.001	0.810 ± 0.001	0.100 ± 0.050	1.220 ± 0.020
February	2.620 ± 0.020	0.430 ± 0.050	0.700 ± 0.001	0.630 ± 0.020

4. Discusion

N. foetida is an important anti-cancer medicinal plant, which possesses various bioactive substances, of which CPT is the most important[8]. CPT from *N. foetida* has been analyzed by HPLC, HPTLC, DESI-MS and 1H-NMR methods[1,5-10]. In present investigation, CPT was quantified by the HPLC method described by Fulzele and Satdive[5]. Among different camptothecin producing plant species, *N. foetida* has the highest CPT accumulation[2,3]. *N. foetida* trees cultivated in north-western agro-climatic region of Jammu, India, accumulated 0.1% dry wt CPT in roots and seeds, whereas bark produced lower concentrations of CPT[7]. In our investigation, the CPT content was highest in roots followed by fruits then in stems and leaves of *N. foetida*. The similar pattern variation in accumulation of CPT is observed[5,11]. The results of the present study also showed that concentration of CPT accumulated in the root of *N. foetida* collected in the month of February was about 13 fold higher in comparison with the roots when collected in October.

Padmanabha *et al*[11] showed the general patterns of accumulation of CPT in *N. foetida* across individuals, plant parts, plant size and sex of plants, in the Western Ghats of India. Similarly Puri *et al*[7] demonstrated that *N. foetida* trees cultivated in north-western agro-climatic region of Jammu, India, accumulated 0.1% dry wt CPT in roots and seeds, whereas bark produced lower concentrations of CPT. The present results showed that concentration of CPT accumulated in the root of *N. foetida* was 2-fold higher in comparison with the fruit of *N. foetida* whereas it is about 3-fold higher in stem and leaves. We also reported that the geographical and climatic conditions have remarkable influence in the content of CPT in *N. foetida*. Different *N. foetida* collected in the month of February from different regions (Mahabaleshwar and Patan regions of Maharashtra state and Sirsi region of Karnataka state) of Western Ghats, India showed variation in CPT accumulation[1]. Our findings of present study are comparable with other research groups working on CPT from *N. foetida* and indicate that the accumulation of CPT differs in different parts of *N. foetida* and the content varies periodically.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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